PLASMODIUM FALCIPARUM malaria represents one of the most important selective factors affecting human populations. Children are most often infected among the 2.5 billion people living in malaria-endemic countries. Frequent mortality among those infected makes malaria a powerful selective influence for factors that confer resistance. These innate factors may provide insights into the evolutionary process and could suggest possible avenues for therapeutic intervention.

There may be factors that confer resistance to malaria during the hepatic stage, but little is known about this portion of the parasite’s life cycle. Several inherited characteristics of red cells (RBCs) lead to resistance at the erythrocytic stage, and the existence of such RBC genetic defects at high frequency is believed to reflect the positive selective pressure of malaria (Fig 1).

The in vitro culture system described just over a decade ago by Jensen and Trager afforded new opportunities to investigate the mechanism by which RBC defects enhanced host resistance to malaria. For the first time, malaria infection of RBCs could be studied independently of the effects of other organs such as the liver and spleen. Reticulocytes and leukocytes could be removed, and the age of RBCs could be controlled. The entire immune system, including antibodies, complement, effector cells, and lymphokines could be eliminated from the culture system if desired. In this way, the influence of intrinsic properties of the RBC membrane, cytoplasm, and especially hemoglobin on malaria infection could be evaluated. Paradoxically, these same strengths of the culture system also underlie its weaknesses. If enhanced resistance to malaria is based on abnormal interactions of infected variant RBCs with complement, macrophages, or the splenic filtration mechanism, the simple in vitro culture system will never discern or elucidate the involved mechanisms. The culture medium commonly used (RPMI 1640) is excessively rich in a variety of nutrients (eg, the initial glucose concentration is >500 mg/dL). Overabundance of crucial nutrients such as iron, paraaminobenzoic acid, or glucose might mask the potential role of limiting amounts in vivo in contributing to resistance to infection. Some investigators have attempted to modify the culture system to address these concerns, but many factors (eg, splenic function) cannot be reproduced in vitro.

Our review of this subject is restricted almost exclusively to P falciparum malaria and focuses on data obtained with the in vitro culture system. We review the genetic RBC abnormalities associated with resistance to malaria and survey the advances made in understanding the molecular and cellular mechanisms involved.

HEMOGLOBIN S

Early epidemiologic and clinical studies suggested that sickle trait individuals become infected with P falciparum, but fewer died of the infection as compared with individuals with normal hemoglobin. Luzzatto et al reported in 1970 that the rate of sickling of deoxygenated parasitized RBCs from ϒ heterozygotes was two to eight times faster than nonparasitized cells in the same blood. These data were interpreted as suggesting that accelerated sickling of parasitized RBCs in vivo might predispose such infected cells to early removal from the circulation, thereby limiting the overall level of parasitemia. However, in these early experiments, total deoxygenation was accomplished with sodium meta-bisulfite, creating conditions very different from those operative in infected patients. Subsequent studies addressed these concerns by using nitrogen to induce deoxygenation. Sickling curves of nonparasitized and early and late parasitized RBCs were defined. Accelerated morphologic sickling occurred in sickle trait cells containing parasite ring forms, an early stage in the erythrocyte phase of the life cycle. Accelerated sickling of cells containing ring forms as compared with nonparasitized sickle trait cells was observed at 50% oxygen saturation. Hence, the accelerated destruction of parasitized RBCs is probably one of the mechanisms by which HbS carriers are afforded protection against P falciparum. Although trophozoite-containing RBCs did not undergo morphologic sickling, HbS polymer was nonetheless detectable by electron microscopy. Low pH, likely to be present in late-stage or trophozoite-infected RBCs would enhance polymerization but might lead to multiple nucleation events without sufficient growth of polymer to induce morphologic sickling.

Other mechanisms may also be involved. Sickle trait cells in 17% oxygen support normal growth of P falciparum. If the oxygen tension is reduced to 3% after two days of growth in 17% oxygen, parasites growing in sickle trait cells die within two to three days. Survival in SS or SC cells is even shorter. These data may be interpreted as follows. Parasitized sickle trait cells that survive despite enhanced sickling during the ring form stage may be compromised during deep vascular schizogony. During this period in the parasite’s life cycle, parasitized RBCs adhere to endothelial surfaces of venules because of trophozoite-induced knob formation on the RBC surface. Venules bedecked with adherent parasitized cells become partially or totally obstructed, leading to hypoxia and low blood pH. Such conditions favor sickling and compromise parasite maturation.

The mechanism of parasite death in sickled AS and SS cells is not fully understood. Friedman et al proposed that loss of K+ induced by sickling could be detrimental to parasite growth due to low intracellular K+ concentrations.
Alternatively, an accompanying loss of water may increase the mean corpuscular hemoglobin concentration (MCHC) and enhance HbS polymerization. The main argument favoring a role for K⁺ and/or cell water loss is the partial abatement of sickling and increased parasite growth observed when AS cells are suspended in high extracellular K⁺. Further investigation has provided data that favor the hypothesis that water loss and enhanced polymer formation are more important than an intrinsic effect of low intracellular K⁺. Ginsburg et al and Tanabe et al showed that ouabain-treated RBCs, enriched in intracellular Na⁺ and depleted of K⁺, sustain the growth of parasites normally.

Polymerized hemoglobin appears to be a poor substrate for the proteases of *P. falciparum*. The HbS polymer may also interfere directly with some critical function of the parasite. Ultrastructural studies by Friedman suggest that AS cells show vacuolization after six hours of deoxygenation. Parasites in SS cells exhibit disruption of the parasitophorous vacuole and other membranes. Such changes could be due to mechanical effects of polymerized hemoglobin or could reflect metabolic disruption induced by sickling.

Several other observations are pertinent to the observed resistance of cells containing HbS to *P. falciparum*. Pasvol et al³ confirmed inhibition of parasite growth in deoxygenated AS cells and also reported a significant decrease in invasion rate under these conditions. The data of Olson and Nagel, obtained using a sensitive method to detect invasion of highly synchronized cultures of AS RBCs, contradicts this last claim. Orji et al⁴ hypothesized that free protoporphyrin IX could be involved in the death of *P. falciparum* in both SS and AS cells. Heme toxicity would not readily explain the deoxygenation dependency of growth inhibition, however. The postnatal decrease in fetal hemoglobin is retarded in sickle trait individuals. Hb F containing cells are poor hosts to plasmodia (described below). Thus, the retarded hemoglobin switch may be of value during the critical first years of life when immune mechanisms are poorly developed.

In summary, during the early stages of infection, parasite-enhanced sickling may lead to preferential destruction of parasitized RBCs. During later stages of *P. falciparum* maturation, parasite-induced RBC membrane changes lead to cell trapping within venules and hypoxia-induced sickling. The parasites within sickled RBCs are apparently killed. These mechanisms are consistent with the observation that sickle trait individuals are not resistant to becoming infected with *P. falciparum* but are less likely to die of their infection than individuals with normal hemoglobin. This mechanism renders the carriers more fit than normal individuals, increasing their chance to reproduce. Their advantage is balanced by the decreased fitness of the homozygote for β⁺, and the gene frequency eventually reaches an equilibrium in the population.

**HEMOGLOBIN C**

A high frequency of the β² gene, found in Central West Africa, has been interpreted to imply that production of HbC confers a selective advantage. Our understanding of the interaction between HbC-containing RBCs and malaria is less complete than for HbS-containing RBCs. HbC has a net increase in positive charge as compared with HbA because of substitution of lysine for glutamic acid at position 6 of the β chain. Friedman et al first described severely decreased growth of *P. falciparum* in oxygenated CC cells. Working with synchronized cultures, Olson and Nagel confirmed this result and demonstrated (a) that *P. falciparum* growth in CC cells is not significantly modified by deoxygenation or suspension in high potassium concentrations to prevent dehydration, (b) that parasitized CC cells are very resistant to lysis as compared with parasitized AA cells, and (c) that degenerative late schizonts are observed on day 4 during the second cycle of infection. This last observation is compatible with the incapacity of parasitized CC cells to complete schizogony with merozoite release. Inability to release merozoites may be related to increased osmotic resistance of CC cells. Impaired release of merozoites may account for the rather flat growth curve characteristic of parasites in HbC cells.
RBCs containing equal amounts of HbA and HbC from heterozygotes for $\beta^c$ behave like normal cells when infected with *P. falciparum* in vitro. These observations appear to be inconsistent with the concept that the high frequency of HbC in certain populations is the result of a balanced polymorphism in which the heterozygote is the advantageous genotypic form. This paradox has not been resolved. Perhaps the culture system is not sensitive enough to detect abnormal parasite growth in HbC trait cells. Alternatively, the selective advantage conferred by these cells may pertain to a portion of the malaria cycle not tested in culture.

The selective pressure to increase $\beta^c$ gene frequency might operate in double heterozygotes for HbS and HbC. This rather novel idea is tenable for several reasons. Although oxygenated cells containing HbC and HbS from double heterozygotes are indistinguishable from normal cells as hosts for plasmodia, parasites rapidly die under low oxygen conditions. SC cells behave like SS cells under these conditions. HbC coexists with HbS in nearly all populations in which the $\beta^c$ gene is common with the exception of the small Dogon population in Mali, in which the $\beta^c$ gene could have been eliminated by genetic drift. In the United States, double heterozygotes for HbS and HbC normally survive beyond the reproductive age. Even under the extreme conditions in sub-Saharan West Africa, doubly heterozygous individuals may have significantly higher fitness than HbS homozygotes. This model could explain the expanded frequency of a second advantageous gene in a population in which one or more genes already provide resistance to malaria. Almost all populations exposed to pandemic malaria exhibit more than one RBC defect that provides protection. More work on the equilibrium and interrelationships of the $\beta^a$, $\beta^c$, and $\beta^e$ genes in Africa will be required before the interaction of the $\beta^a$ and $\beta^c$ genes in double heterozygotes can be accepted as the basis for the high frequency of the $\beta^c$ gene.

**HEMOGLOBIN E**

The gene for HbE ($\beta^{26}$ Glu $\rightarrow$ Lys) is very frequent in Southeast Asia. Epidemiologic studies have long suggested a causal connection between the high frequency of this abnormal hemoglobin and malaria. The $\beta^e$ gene may have arisen first among the Khmer people because its frequency is 55% in Sarim and Angkor Wat. The frequency of HbE decreases concentrically around the areas where the frontiers of Cambodia, Laos, and Thailand adjoin. The outer edges of the geographic area in which HbE is found involve Bangladesh, Assam, and Madagascar to the west and South China, Indonesia, and the Philippines to the east. The $\beta^e$ gene is only one of several genes causing defective RBCs that are frequent in Southeast Asia. For example, in central regions of Indochina, RBC genetic defects including HbE, $\alpha$ and $\beta$ thalassemia, and G6PD deficiency are so frequent that calculations suggest that only 15% of the population has "normal" RBCs.

Nagel et al. first demonstrated a moderate decrease in growth of *P. falciparum* in cells from homozygotes (EE) but normal growth in cells from heterozygous individuals (AE). A subsequent study reporting failure to show retarded growth of *P. falciparum* in EE RBCs may be criticized on methodologic grounds. The apparent conflict between these two studies was resolved when Vernes et al. in a thorough study, reported retarded growth of the parasite in both EE and AE cells. Growth retardation was more marked in cells from homozygous individuals. Growth retardation was higher under oxygenated conditions (20% $O_2$) than in a low oxygen environment (5%). Vitamin C enhanced growth of *P. falciparum* in EE cells cultured under 20% oxygen. This result is interesting in the context of the properties of HbE, a somewhat unstable hemoglobin that may induce oxidative damage to parasites by generating free radicals.

Significantly higher levels of antimalarial antibodies and lower parasitemia have been found in carriers of HbE as compared with AA individuals from the same areas. In addition, Bunyaratieje et al. reported that parasitized EE and AE RBCs are phagocytized more readily by human monocytes than are infected normal RBCs. The levels of phagocytosis observed were low, and these data have not yet been confirmed by other investigators. Nonetheless, this intriguing observation, if correct, would suggest that the surface of HbE-containing RBCs is modified differently by the parasite than are membranes of normal RBCs.

**HEMOGLOBIN F**

The role of HbF is particularly important in carriers of $\beta^c$, $\beta^e$, and $\beta$-thalassemia genes who exhibit a significant retardation in the fetal to adult switch during the first 5 years of life. The effect of HbF may account for the decreased parasitemia observed in infected infants during the first 6 months of life and may afford protection to individuals with abnormal globin genes before immune-mediated resistance becomes effective. Pasvol et al. found that *P. falciparum* growth is retarded in cord blood cells containing ~85% HbF, in RBCs that contain 20% HbF obtained from infants, and in RBCs containing 100% HbF from adult homozygotes for hereditary persistence of fetal hemoglobin (HPFH). Retardation of growth in all of these cells implicates HbF rather than RBC age or morphological differences. Retardation of parasite growth in cells containing HbF is observed in medium 199 that is relatively deficient in reduced glutathione but not when parasites are grown in RPMI 1640 at low $O_2$ (5%). HbF may increase oxidative stress, accounting for retarded parasite growth, although this hypothesis has not been tested directly.

**THE THALASSEMIAS**

The thalassemias, disorders of globin synthesis, are genetically and physiologically more complex than hemoglobinopathies and enzyme deficiencies. Among individuals with $\beta$-thalassemia, the deficiency of $\beta$-chain synthesis ($\beta^0$ or $\beta^+$) and levels of HbA$_2$ and F vary. The clinical spectrum of $\alpha$-thalassemia includes a clinically silent form ($-\alpha/\alpha\alpha$), a form with slight or moderate anemia and RBC hypochromia and microcytosis ($-\alpha/\alpha$ and $-\alpha/\alpha\alpha$), a form with moderate to severe anemia ($-\alpha/-\alpha$), and finally a form lethal...
in utero or at birth (−−/−−). In one form (−α/−α), an abnormal hemoglobin (HbH-δ4) is produced that is both unstable and a poor O2 transporter because of high affinity and low cooperativity.

Various forms of thalassemia are very common throughout the Mediterranean basin, in Africa and in Southeast Asia. A number of early population studies, summarized by Weatherall and Clegg,36 support a selective advantage for β-thalassemia genes in the Mediterranean region. A more recent review also summarizes data supporting resistance conferred by thalassemia genes.34 Important population surveys have continued during the past decade. Flint et al35 showed that the frequency of α-thalasemia, as defined by DNA analysis, but not of other unlinked DNA polymorphisms, exhibited an altitude and latitude-dependent distribution that correlated with the presence of malaria endemicity throughout Melanesia.

Development of the malaria culture system allowed study of thalassemic RBCs in vitro. Friedman reported normal growth in β-thalassemic trait red cells, but the parasites were more susceptible to oxidants than were parasites growing in normal cells.32 Pasvol and Wilson36 were also unable to demonstrate inhibited parasite growth in thalassemic RBCs under optimal culture conditions, and similar results were obtained by Roth et al37 in β-thalassemic trait RBCs from Sardinia. A clear-cut diminution in growth rate was observed in hemoglobin H-containing RBCs, but no decrease in proliferation was detected in the RBCs of individuals lacking one or two α-globin genes.38

Poor parasite growth in HbH or Hb Constant Spring-containing RBCs was also reported by Yuthavong et al.39 In addition, the infected variant RBCs were more readily phagocytosed than normal infected cells. This type of finding underscores the possibility that variant RBCs may impair parasite survival by extrinsic mechanisms which cannot be detected in a culture system (eg, variant RBCs might offer protection to the host by rendering passage of infected cells through the spleen difficult or impossible, thus assuring death of parasites by phagocytosis).

Manipulation of culture conditions may elucidate differences in the growth of parasites in cells from individuals with various types of thalassemia. Brockelman et al46 replaced 90% of the usual culture medium (RPMI 1640) with minimum essential medium (MEM, Sigma Corp, St Louis) and were able to show impaired growth in thalassemic RBCs. The investigators noted that MEM-diluted medium contains lower concentrations of certain amino acids required by the parasites. According to their hypothesis, the parasites are unable to acquire adequate amounts of these nutrients by digestion of hemoglobin because of the low hemoglobin content of thalassemic RBCs. This effect could be masked in undiluted RPMI 1640 medium. Indeed, many differences exist between RPMI 1640 and MEM. The latter lacks alanine, aspartate, asparagine, glutamate, hydroxyproline, proline, and serine, but contains more histidine, threonine, tyrosine, and valine. In addition, biotin, paraaminobenzoic acid, and cobalamin are also lacking in MEM but exist in RPMI 1640. Much work will be required to pinpoint the crucial nutrient(s) responsible for relative retardation of parasite growth in thalassemic RBCs.

The findings summarized above have led to several theories concerning the manner in which deficient globin production in thalassemia RBCs may retard parasite growth. These include (a) intraerythroid iron deficiency in thalassemia,41 (b) interacting nutritional deficiencies,42 (c) increased susceptibility to oxidant stress,43 (d) increased vulnerability to cell-mediated damage (phagocytosis),44 (e) elevation and persistence of fetal hemoglobin in infancy and early childhood,53,45 (f) associated low pyridoxine-phosphate oxidase activity in thalassemias,46 and (g) low hemoglobin content.41 Future work in vitro will be concentrated on dissecting out these many possible mechanisms.

Population surveys are useful, although such studies cannot elucidate the mechanism for protection of thalassemic individuals from malaria. Willcox et al,47 in a recent survey in Northern Liberia, showed that there was an increasing frequency of β-thalassemia trait with increasing age. Classically, this type of age-dependent gene frequency suggests a selection in favor of the thalassemic carriers. In addition, by using the criterion of parasite density (parasitemia > 1 x 105/L) to indicate probability of lethal infection, the relative risk of lethal infection was calculated as 0.45 (upper 95% confidence limit = 0.79) in children aged 1 to 4 years. These data provide further evidence for the protective effect of thalassemia against malaria. A better understanding of the mechanisms involved is needed (eg, the role of HbF has not been fully evaluated). Field studies designed to seek a correlation between the rate of decrease of HbF during the first 5 years of life with the risk of fatal malaria would be very useful.

MUTATIONS IN RBC SURFACE ANTIGENS

The pioneering discovery of the role of Duffy blood group determinants in the erythrocyte invasion by Plasmodium vivax has opened a new avenue to understanding of RBC resistance to malaria.48,49 A significant proportion of African and African Americans are entirely resistant to Plasmodium vivax malaria.50 Many such individuals are Duffy (−),51 a trait that is rare elsewhere and observed only in ethnic groups that have admixed with Blacks (Arabs, Oriental Jews, Druzes52). The Duffy blood group system is polymorphic and includes five alloantigens. Two Mendelian alleles (Fya and Fyb) map to chromosome 1. The Duffy (−) characteristic corresponds to lack of expression of both Fya and Fyb antigens (Fya−b−). Miller et al48 first demonstrated that Fya−b− erythrocytes are resistant to invasion by Plasmodium knowlesi merozoites in vitro. This plasmodium is closely related to Plasmodium vivax. The next step was to demonstrate that Duffy (−) individuals were resistant to Plasmodium vivax malaria. Because Plasmodium vivax cannot be propagated in culture, this experiment could be performed only in human volunteers. Duffy (−) individuals were shown to be resistant to infection when exposed to mosquitoes infected with Plasmodium vivax, whereas all other volunteers became ill 11 to 15 days after exposure.49 These findings explain the very high frequency of the Duffy (−) phenotype in parts of
West Africa. Although *P. vivax* is not intrinsically a life-threatening disease, it can contribute to infant mortality by negative interaction with malnutrition and other infections.

Alloantibodies to *Fy* α partially block erythrocyte invasion by *P. knowlesi*. Furthermore, Nichols et al.19 described a new specificity (Fy6) in the Duffy system detected with a murine monoclonal antibody (MoAb). The epitope involved may very well reside on the Fyα determinant as it correlates with *P. knowlesi* invasion. Western blotting has demonstrated that both Fyα and Fy6 reside in a glycoprotein of ~46,000 molecular weight (mol wt).33,34

The Duffy system may be prototypical for receptors involved in invasion of RBCs by plasmodial merozoites. Receptors for invasion by *P. falciparum* merozoites are believed to be members of the glycoporin system. The exact role of each glycoporin (α, β, and γδ) has not been defined, and the methodology used in implicating these as plasmodial receptors has been criticized. Erythrocyte band 3 may also function as a receptor. Cells showing complete absence of A or α glycoporin (Ena-) have a significantly lower rate of invasion by *P. falciparum* than normal cells.55 Only about ten individuals are known to carry this genotype. Surprisingly, absence of glycoporin A has no apparent effect on RBC function. The S-s-U-genotype is present at higher frequencies in Africa, compatible with balanced polymorphism.56 Pasvol57 reported a 50% to 75% reduction in the invasion rate by *P. falciparum* merozoites into such cells. The early report by Pasvol et al.57 that *W*α- RBCs, also polymorphic in Africa, are resistant to invasion has not been confirmed.58 RBCs expressing α-δ hybrids (Lepore-like) of the genes specifying α and δ glycoporins, show a 50% reduction in invasion.55 Tn RBCs are incompletely glycosylated,59 exposing a cryptic epitope that can be identified serologically; such cells are also resistant to invasion.60

*M^4^* erythrocytes that lack A and B glycoporins have been tested with two *P. falciparum* strains, 7G8 and Camp.61 The former invaded with 50% efficiency, whereas the latter invaded at only 20% the rate of control. These results raise the possibility of heterogeneity among *P. falciparum* merozoite variants with respect to their RBC binding determinants. That neuraminidase treatments can inhibit invasion has been interpreted to mean that sialic acid may be involved in interaction with the parasite. Caution is advisable in the interpretation of these experiments. Enzyme treatment could induce conformational changes in the glycosylated protein. Mitchell et al.68 exposed Tn cells to three different strains of *P. falciparum* (Camp, Thai-1, Thai-2) before and after treatment with neuraminidase. Heterogeneity of receptors on the RBC surface was believed to explain the results best.

All described glycoporin mutants only reduce invasion; they do not block it. These observations suggest that the receptor site for *P. falciparum* is complex and multivalent, with differences in affinity constants among redundant sites. Preventing malaria infection by blocking receptors used during RBC invasion may be difficult. Since invasion studies have been performed in only a few donors, confusion might be introduced if the RBCs studied had undiscovered defects in addition to the mutation in glycoporin.

**CYTOSKELETON ABNORMALITIES**

The malaria invasion process is complex and involves several stages. These include the initial nonspecific attachment, reorientation of the polarity of the merozoite, RBC membrane flapping, and the zipper-type actual introduction of the merozoite into the RBC.61 Cytoskeleton proteins must be active participants in this process, and mutations of these proteins could generate RBC resistance to *P. falciparum*.

Ovalocytosis, a morphologic variant due to an RBC cytoskeletal abnormality, is present in ~30% of the Melanesian population of Papua New Guinea and other aboriginal populations of Southeast Asia.64,65 Only ovalocytosis among RBC cytoskeletal defects has such a high polymorphic frequency. Ovalocytes have smaller axial ratios than elliptocytes. The thermal deformation profile of ovalocytes, like elliptocytes, is abnormal.66 Expression of surface blood groups is altered57 and deformability of these cells is diminished.64,65 Individuals with ovalocytosis do not have hemolysis. The molecular defect(s) in ovalocytosis has not been defined.

Epidemiologic evidence suggests that individuals with ovalocytosis have lower parasitemia than normal subjects when infected with *P. falciparum, P. vivax*, or *P. malariae*.70 Ovalocytes are highly resistant to invasion by both *P. falciparum*64 and *P. knowlesi*71 merozoites. These parasites do not bind to the same receptor, suggesting that resistance may be due to some property of the ovalocytes’ membrane.

Elliptocytes, secondary to glycoporin γ or band 4.1 deficiency, have also been reported to have resistance to invasion by both *P. knowlesi* and *P. falciparum*.72 Elliptocytes have cytoskeleton abnormalities that reduce vertical interactions involved in anchoring the cytoskeleton core to proteins imbedded in the lipid bilayer and have abnormal deformability. The presence of decreased invasion by two types of plasmodia suggest that this defect alters a later stage of invasion than receptor recognition. More recently, Schulman et al.73 reported decreased parasite growth in direct proportion to the extent of spectrin deficiency in hereditary spherocytosis. Shear et al.74 showed dramatically decreased growth by *P. berghei* and *P. chabaudi* in the RBCs of ankyrin/ spectrin-deficient mice.

Because the invasion process is a complex sequence of stages, considerable information could be gleaned from studies defining the particular stage in which each of these invasion-inhibiting defects is operating. The technology for observing and recording each stage has been developed, but it has been applied to study only a few of the RBC membrane defects.75

**GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY**

When the subject of G6PD was last reviewed by Luzzatto a decade ago,76 extensive epidemiologic evidence but few direct experimental data supported the concept that G6PD deficiency was protective in the presence of endemic falciparum malaria. Luzzatto had proposed that it was the heterozygous female, particularly of the genotype *Gd^b^*/Gd^a^*, on
whom protection was conferred. This hypothesis was supported by two sets of data. Because of lyonization of the X chromosome, RBCs of heterozygous females are of two types, either GdA- or GdA+. Parasites were rarely found in GdA- RBCs in an infected African female mosaic of the GdA/GdA- genotype.77 A survey of infection rates among African children of different G6PD genotype provided additional data that supported Luzzatto’s hypothesis.78

In the late 1970s, direct experimental evidence from the malaria culture system became available. Friedman79 demonstrated that G6PD-deficient RBCs, probably of the G6PD A- type, support normal parasite growth under ordinary culture conditions. However, when oxidative stress was generated by use of menadione or riboflavin, the parasites growing in G6PD-deficient RBCs were more readily inhibited than in those normal cells. Subsequently, and in contrast, Roth et al.77 studying Sardinian patients with G6PD Mediterranean, showed a clear-cut inhibition of growth even under optimum conditions of culture in candle jars, although retarded growth was observed during only three growth cycles. These results were in agreement with those of Luzzatto et al.,78 who also established that invasion of G6PD-deficient RBCs proceeded normally; it was intracellular growth that was impaired. Luzzatto et al also made the important discovery that parasites appeared to adapt and normalize their growth after four to five cycles in G6PD-deficient cells. The explanation for this adaptive ability was not long in coming. The existence of a parasite gene for G6PD had been correctly predicted by Luzzatto a decade ago.77 Hempelmann and Wilson80 found evidence suggesting that infected RBCs had an electrophoretically unique protein with G6PD activity. In a subsequent study, Usanga and Luzzatto81 documented the presence of a parasite-encoded G6PD which became detectable as the adaptation to growth in G6PD-deficient RBCs progressed. Yoshida and Roth82 characterized the kinetics of a parasite-derived G6PD from the A2 clone of FCR-3 P. falciparum, grown in G6PD Mediterranean RBCs with very low human G6PD activity. The parasite-encoded enzyme has significantly higher affinities for the substrates glucose-6-phosphate and NADP+ than the human counterpart. A human G6PD cDNA probe strongly hybridized with sequences in the parasite genome.83 Work has begun on the cloning of the parasite G6PD gene.84

The hypothesis that female heterozygotes for G6PD deficiency are protected from lethal malaria infections has been further developed based on knowledge of the parasite-encoded G6PD. In such heterozygotes, parasites may invade either Gd-normal or Gd A- RBCs during successive cycles. The stimulus to the parasite to adapt by G6PD gene activation would be attenuated significantly.84 Male hemizygotes who are completely deficient in G6PD may have a smaller but perhaps significant advantage. Retarded growth of the parasite for even three to five cycles (six to ten days) during adaption by G6PD gene activation may decrease morbidity and mortality.

The usual role of G6PD in providing NADPH as a defense against oxidative stress with consequent glutathione oxidation may not be the crucial task performed by the parasite G6PD. The quantity of parasite-encoded enzyme that is induced is small compared with the activity found in normal, uninfected RBCs.84 Lysates of adapted parasites grown in G6PD-deficient RBCs contain no more than 15% to 20% of the activity of normal, uninfected RBCs with respect to G6PD-dependent glutathione reduction. Unadapted parasites growing in G6PD-deficient RBCs are eight times more sensitive to oxidative stress induced by acetylsalicylic acid (ASA) than parasites grown in normal RBCs.85 Pre-adaptation by growth in G6PD-deficient RBCs increased resistance to APH only modestly. This small gain in resistance to oxidative stress supports the notion that there may be an entirely different role for the low levels of G6PD induced during parasite adaptation. Nevertheless, oxidative stress remains a major hypothesis for G6PD deficiency-dependent mechanism of malaria resistance (e.g., excessive release of ferriheme has been observed when G6PD-deficient RBC are exposed to an oxidative stress in the form of menadione86; ferriheme is toxic to malaria parasites87).

The interaction between favism and G6PD deficiency is fascinating but difficult to investigate because of its seasonal occurrence. Favism is observed exclusively in the Mediterranean area and the Near East but is virtually unknown among Africans in whom G6PD gene frequencies can be very high. The involvement of a separate, interactive mutation should be considered. Efforts have been made to use the culture system to investigate favism. Isouramib, an oxidant isolated from fava beans, was found to be more damaging to parasites in G6PD-deficient RBCs than to parasites in normal cells.87 Increased formation of membrane disulfides was associated with poor parasite growth.88 Divicine, another fava bean alkaloid, possesses antimalarial activity in vivo against P. vinckei in mice.89 Desferrioxamine blocks the hemolytic and antimalarial effect of this compound. These studies suggest a number of pharmacologic approaches to malaria therapy but may not be relevant to the interacting evolution of P. falciparum and RBC G6PD deficiency.

During the past decade, the techniques of in vitro culture and molecular biology have demonstrated clearly and directly that G6PD deficiency is inhibitory to parasite growth. Demonstration that the malarial parasite can synthesize G6PD provides an explanation, although a complex one, for the success of enzyme deficiency as a defense against malaria. The actual biochemical mechanism underlying this resistance remains to be elucidated. The enhanced susceptibility of parasites in G6PD-deficient RBCs to oxidative stress remains a leading hypothesis but has not been definitively tested. We should soon know the nature of the parasite gene and its properties in comparison to the human G6PD gene. Clarification of the role of G6PD in the rapidly growing erythrocytic stages of P. falciparum may also be anticipated.

**SUMMARY**

The study of inherited RBC resistance to malaria has increased our knowledge of the biochemistry and physiology of the host–parasite interaction and suggested potential sites for therapeutic intervention. Discovery by Jensen and Trager1 of the in vitro culture system for P. falciparum has
facilitated research in this area. Known RBC defects may affect invasion, growth, or merozite liberation (Fig 1). Significant advances made in understanding mechanisms underlying protection against malaria should not obscure the fact that the data are far from complete. More knowledge is needed about the influence of the erythrocyte cytoskeleton on invasion and growth of parasites as well as the potential role of phospholipids, erythrocyte enzymes other than G6PD, or other metabolic products. Application of DNA analysis and recombinant technology may have an increasing impact on study of the interaction of RBC defects with malarial parasites.

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